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A new species of Chinese horned toad (Anura, Megophryidae, *Boulenophrys*) from western Guangdong Province, China, with recommendations for extinction risk assessments for several related congeners

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Abstract

The genus *Boulenophrys* is predominantly distributed in China and exhibits high species diversity. However, accurate conservation assessments for this genus are hindered by its unresolved taxonomy, which is linked to morphological conservatism, sympatric distribution patterns, and limited field survey data. In this study, we describe a new species, namely *Boulenophrys yangchunensis* **sp. nov.**, and report new locality records for *B. acuta* (Wang, Li & Jin, 2014) and *B. yunkaiensis* Qi, Wang, Lyu & Wang, 2021 from western Guangdong Province, China. Additionally, we propose downlisting *B. acuta*, currently listed as Critically Endangered (CR) on the IUCN Red List of Threatened Species, to a lower threat category, and we recommend an extinction risk category update for *Boulenophrys yangchunensis* **sp. nov.**, *B. yunkaiensis*, and *B. gaolanensis* Song, Wang, Qi, Wang & Wang, 2024. We further discuss key conservation challenges and provide targeted management recommendations to address these challenges.

Key Words

conservation assessment, management suggestions, molecular phylogeny, morphological conservatism, range extension, reassessment, sympatry, systematics

Introduction

The speciose genus *Boulenophrys* Fei, Ye & Jiang, 2016, known as the Chinese horned toads and currently comprising 78 recognized species, is widespread in the subtropical and tropical areas of mainland East Asia (Frost 2025). Specifically, the genus is principally distributed in southern China and southwards into northernmost Vietnam, Laos, Myanmar, and Thailand (Fei and Ye 2016; Lyu et al. 2023; Frost 2025). *Boulenophrys* possess limited dispersal abilities and narrow ecological niches, resulting in restricted distribution ranges for many species

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(Wang et al. 2019a; Lyu et al. 2023). Due to the combined effect of various factors, conservation efforts for this genus have encountered significant challenges. Besides, their high levels of morphological conservatism and the lack of follow-up surveys have led to inadequate protection due to misidentifications and missing data (Lin et al. 2024). Therefore, accurate assessments of the extinction risk for *Boulenophrys* species still require a significant amount of field surveys and continuous data updates.

As the research progressed, Guangdong Province, China, gradually became one of the provinces with extremely high diversity of the genus *Boulenophrys* (20 of 77 species). Currently, the type locality of 15 species is located within the jurisdiction of Guangdong Province, i.e., *B. acuta* (Wang, Li & Jin, 2014), *B. dongguanensis* (Wang & Wang, 2019), *B. elongata* Zeng, Wang, Chen, Xiao, Zhan, Li & Lin, 2024, *B. fengshunensis* Wang, Zeng, Lyu & Wang, 2022, *B. gaolanensis* Song, Wang, Qi, Wang & Wang, 2024, *B. hungtai* Wang, Zeng, Lyu, Xiao & Wang, 2022, *B. insularis* (Wang, Liu, Lyu, Zeng & Wang, 2017), *B. nankunensis* (Wang, Zeng & Wang, 2019), *B. nanlingensis* (Lyu, Wang, Liu & Wang, 2019), *B. obesa* (Wang, Li & Zhao, 2014), *B. pepe* Wang & Zeng, 2024, *B. puningensis* Wang, Zeng, Lyu, Xiao & Wang, 2022, *B. shimentaina* (Lyu, Liu & Wang, 2020), *B. yingdeensis* Qi, Lyu, Wang & Wang, 2021, and *B. yunkaiensis* Qi, Wang, Lyu & Wang, 2021 (Lyu et al. 2023; Song et al. 2024; Frost 2025). Among them, the Fengkai horned toad, *Boulenophrys acuta*, was most recently assessed for the IUCN Red List of Threatened Species in 2019 and listed as Critically Endangered (CR) under criteria B1ab(iii), due to its limited distribution range within Heishiding Nature Reserve in Fengkai County, western Guangdong Province, China (Li et al. 2014). Based on molecular data, Song et al. (2024) provided a range extension by reporting a new population from Mt. Yunkai in Xinyi City, western Guangdong Province, China, approximately 155 km away in straight-line distance. During our field surveys in Ehuangzhang NR in Yangchun City, western Guangdong Province, China, we collected a series of sympatric tadpole samples and adult specimens of the genus *Boulenophrys* (Fig. 1). Subsequent molecular analysis revealed that two of the samples (GEP asp062 and GEP asp064; Environmental Protection Engineering for the abbreviation GEP and amphibian tissue sample for the abbreviation asp) clustered with the typical lineage of *B. acuta* with shallow genetic divergence, and four samples (GEP asp061, GEP asp063, and GEP asp065–066) clustered with the typical lineage of *B. yunkaiensis* with shallow genetic divergence. These clusters represent new geographical subpopulations of the two species, respectively (Fig. 2). Additionally, five specimens (GEP a168–170, SYS a009518, and CIB 121751; Chengdu

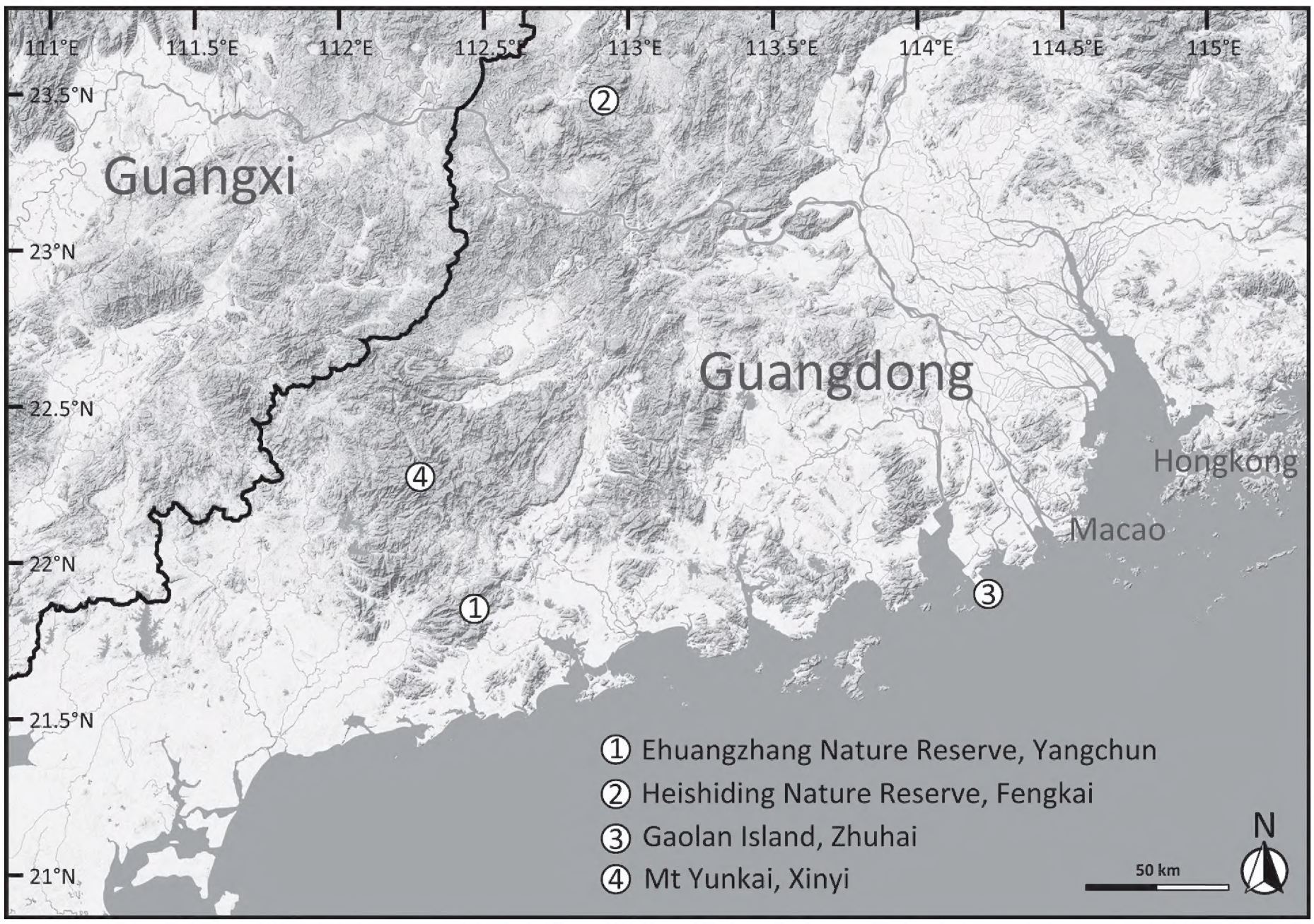

Figure 1. Map showing the type locality of *Boulenophrys yangchunensis* sp. nov. and the new distribution sites of *B. acuta* and *B. yunkaiensis* (1); the type locality of *B. acuta* (2); the type locality of *B. gaolanensis* (3); and the type locality of *B. yunkaiensis* and the new distribution site of *B. acuta* (4).

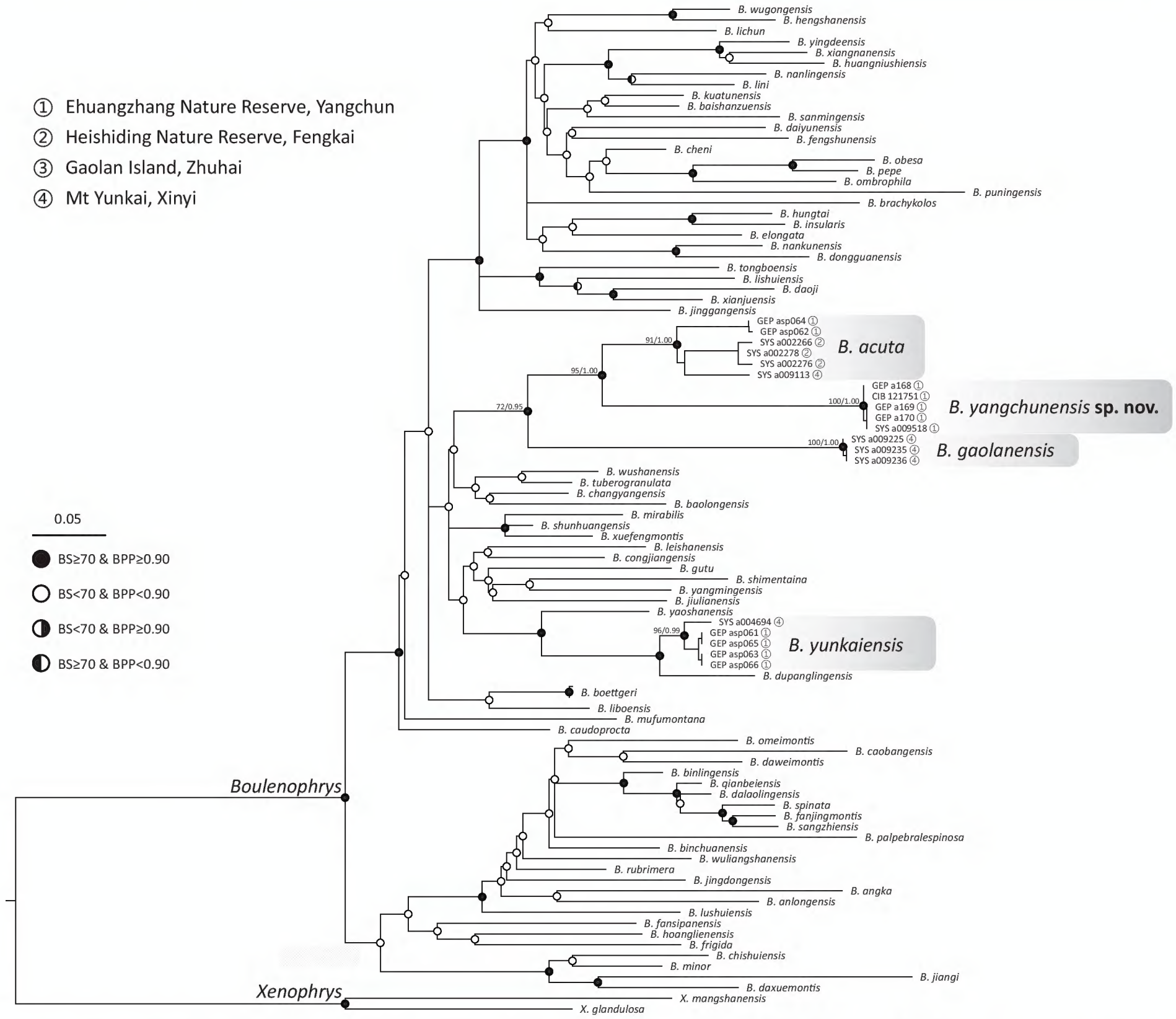

Figure 2. Maximum likelihood tree results from partial DNA sequences of the mitochondrial 16S rRNA and COI gene. Numbers before slashes indicate bootstrap support (BS), and numbers after slashes indicate Bayesian posterior probabilities (BPP).

Institute of Biology, Chinese Academy of Sciences for the abbreviation CIB) exhibited distinct morphological differences and significant genetic divergence from all known congeners within the genus. Therefore, we describe a new species from Ehuangzhang NR, report range extensions for *B. acuta* (approximately 188 km in straight-line distance from its type locality in Heishiding NR) and for *B. yunkaiensis* (approximately 52 km from its type locality in Mt. Yunkai), and also provide a reassessment of the endangered status of *B. acuta* and assessments of *B. yunkaiensis* and *B. gaolanensis*.

Materials and methods

Morphology

Five adult male specimens (GEP a168–170; CIB 121751; SYS a009518) of the genus *Boulenophrys* were collected on 11 August 2023 from Guangdong Yangchun Ehuangzhang Provincial Nature Reserve (21.851912°N, 111.421553°E; ca. 1,060 m a.s.l.) in Yangchun City, Guangdong Province, China. All examined specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol. All studied specimens have been deposited at the Guangdong Polytechnic of Environmental Protection Engineering (GEP) facility in Foshan, Guangdong Province; the Museum of Biology, Sun Yat-sen University in Guangzhou, Guangdong Province, China (SYS); and the Herpetological Museum at the Chengdu Institute of Biology, Chinese Academy of Sciences (CIB) in Chengdu, Sichuan Province, China.

External measurements were recorded with a digital caliper (Neiko 01407A stainless steel 6-inch digital caliper) to the nearest 0.1 mm. These measurements are as follows: SVL (snout–vent length, from tip of snout to posterior margin of vent); HDL (head length, from tip of snout to the articulation of the jaw); HDW (head width, head width at the commissure of the jaws); SNT (snout length, from tip of snout to the anterior corner of the eye); IND (internasal distance, distance between nares); IOD (interorbital distance, minimum distance between

upper eyelids); ED (eye diameter, from the anterior corner of the eye to the posterior corner of the eye); TD (tympanum diameter, horizontal diameter of tympanum); TED (tympanum–eye distance, from anterior edge of tympanum to posterior corner of the eye); HND (hand length, from the proximal border of the outer palmar tubercle to the tip of digit III); RAD (forearm or radioulna length, from the flexed elbow to the proximal border of the outer palmar tubercle); FTL (foot length, from distal end of shank to the tip of digit IV); TIB (crus or tibiofibula length, from the outer surface of the flexed knee to the heel). Sex was determined by external secondary sexual characters, such as the presence of vocal sacs, nuptial pads, or spines in males and their absence in females (Fei et al. 2009). Morphological characters of all recognized species of the genus *Boulenophrys* used for comparisons were based on an examination of museum specimens listed in Appendix I and information available in the literature (Table 1).

Phylogeny

Five tissue samples were derived from the five adult specimens (GEP a168–170; CIB 121751; SYS a009518). Another six tissue samples (GEP asp061–066) were derived from the tail fins of different tadpoles from Guangdong Yangchun Ehuangzhang Provincial Nature Reserve (21.851912°N, 111.421553°E; ca. 1,060 m a.s.l.), and the tadpoles were not collected. In total, 94 samples were used for the phylogenetic analyses, encompassing 11 newly sequenced individuals and others downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Two samples of the genus *Xenophrys* Günther, 1864, were used as outgroups (Suppl. material 1). Two partial mitochondrial genes, the 16S ribosomal RNA (16S) and the cytochrome c oxidase 1 (COI), were used for phylogenetic analysis. DNA extraction, PCR amplification, and sequencing protocols followed Liu et al. (2018).

We used Clustal X 2.0 (Thompson et al. 1997) to align the sequences using default parameters. We then used PartitionFinder (Lanfear et al. 2012) to determine the optimal partitioning schemes, and we determined that partitioning by gene was optimal for 16S, while partitioning by codon position was optimal for COI, with GTR+I+G identified as the best-fit nucleotide substitution model for all partitions. We constructed the phylogenetic trees using maximum likelihood (ML) implemented in RAxMLGUI v.1.3 (Silvestro and Michalak 2012) and Bayesian inference (BI) using MrBayes v.3.2.4 (Ronquist et al. 2012). For the ML analysis, an optimal tree was obtained, and branch supports were evaluated with 1000 rapid bootstrapping replicates. For the BI analysis, two independent runs were conducted, each running for 10,000,000 generations and sampled every 1000 generations, with the first 25% of samples discarded as burn-in, resulting in a potential scale reduction factor (PSRF) of < 0.01. Then, the remaining trees were used to create a consensus tree. Nodes having ML bootstrap values (BS) ≥ 70 and BI posterior probabilities (BPP) ≥ 0.90 were considered well supported. Genetic distances among all *Boulenophrys* samples were calculated in MEGA 11 using the uncorrected *p*-distance model based on the 16S.

Threat assessments

Among the focal species in this work, *B. acuta* has been assessed as Critically Endangered (ASG 2021), but *B. yunkaiensis* and *B. gaolanensis* have not been assessed. Here, the threat assessment of *B. gaolanensis* is relevant, as it is closely related to the taxa described in this manuscript. The geographic area of this study is characterized by low-elevation hills and mountains interrupted by low-elevation agricultural plains and large urban areas. As a result, the populations are disconnected both because of the landscape and human activities. To suggest an extinction risk assessment for these four species, we followed the IUCN Red List categories and criteria (www.iucnredlist.org/resources/redlistguidelines) due to their robustness, especially for species with restricted ranges (Maes et al. 2015), and because evaluations are conducted against quantitative thresholds for five criteria (IUCN 2012). However, we could not use criteria A (population size reduction), C (small and declining population size), and E (quantitative analysis of extinction risk) due to the absence of data on population sizes. Instead, we relied on criteria B (geographic range size) and D (very small population and/or restricted distribution) for the assessments. To determine the extent of occurrence (hereafter EOO) and the area of occupancy (hereafter AOO) for each species, we used the online platform GeoCAT (www.geocat.iucnredlist.org).

Results

Molecular phylogenetic analysis

The BI and ML phylogenetic trees resulted in essentially identical topologies (ML phylogenetic topology in Fig. 2). In the phylogenetic result, the *Boulenophrys* samples from Ehuangzhang NR, Yangchun, formed three independent clades. Among them, samples (GEP asp062 and GEP asp064) from two uncaptured tadpoles clustered with the typical series of *B. acuta* from Heishiding NR, Fengkai, and that from Mt. Yunkai (Song et al. 2024), Xinyi, with good support (BS = 74; BPP = 0.95). In addition, samples (GEP asp061, GEP asp063, and GEP asp065–066) from four uncaptured tadpoles clustered with the typical series of *B. yunkaiensis* from Mt. Yunkai, Xinyi, with strong support (BS = 97; BPP = 0.99), and showed very shallow divergence. Thus, *B. acuta* and *B. yunkaiensis* were

Table 1. Literature for morphological characters of 78 recognized species of *Boulenophrys*.

ID	*Boulenophrys* spp.	References
1	*B. acuta* (Wang, Li & Jin, 2014)	Lyu et al. 2023
2	*B. angka* (Wu, Suwannapoom, Poyarkov, Pawangkhanant, Xu, Jin, Murphy & Che, 2019)	Wu et al. 2019
3	*B. anlongensis* (Li, Lu, Liu & Wang, 2020)	Li et al. 2020; Lyu et al. 2023
4	*B. baishanzuensis* (Wu, Li, Liu, Wang & Wu, 2020)	Wu et al. 2020
5	*B. baolongensis* (Ye, Fei & Xie, 2007)	Ye et al. 2007; Fei and Ye 2016
6	*B. binchuanensis* (Ye & Fei, 1995)	Lyu et al. 2023
7	*B. binlingensis* (Jiang, Fei & Ye, 2009)	Fei et al. 2009; Lyu et al. 2023
8	*B. boettgeri* (Boulenger, 1899)	Lyu et al. 2023
9	*B. brachykolos* (Inger & Romer, 1961)	Lyu et al. 2023
10	*B. caobangensis* (Nguyen, Pham, Nguyen, Luong & Ziegler, 2020)	Nguyen et al. 2020
11	*B. caudoprocta* (Shen, 1994)	Shen 1994; Lyu et al. 2023
12	*B. congjiangensis* (Luo, Wang, Wang, Lu, Wang, Deng & Zhou, 2021)	Lyu et al. 2023
13	*B. changyangensis* Li, Shi, Liu, Zhao, Gao & Wang, 2025	Li et al. 2025
14	*B. cheni* (Wang & Liu, 2014)	Lyu et al. 2023
15	*B. chishuiensis* (Xu, Li, Liu, Wei & Wang, 2020)	Lyu et al. 2023
16	*B. daiyunensis* (Lyu, Wang & Wang, 2021)	Lyu et al. 2023
17	*B. dalaolingensis* Wang, Chen & Liu, 2025	Wang et al. 2025a
18	*B. daoji* (Lyu, Zeng, Wang & Wang, 2021)	Lyu et al. 2023
19	*B. daweimontis* (Rao & Yang, 1997)	Rao and Yang 1997
20	*B. daxuemontis* Liu, Li, Cheng, Wei, Wang & Cheng, 2025	Liu et al. 2025
21	*B. dongguanensis* (Wang & Wang, 2019)	Lyu et al. 2023
22	*B. dupanglingensis* Xiao & Mo, 2025	Xiao et al. 2025
23	*B. elongata* Zeng, Wang, Chen, Xiao, Zhan, Li & Lin, 2024	Zeng et al. 2024
24	*B. fengshunensis* Wang, Zeng, Lyu & Wang, 2022	Lyu et al. 2023
25	*B. fanjingmontis* (Zhang, Liang, Ran & Shen, 2012)	Lyu et al. 2023
26	*B. fansipanensis* (Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018)	Tapley et al. 2018a
27	*B. frigida* (Tapley, Cutaja, Nguyen, Portway, Mahony, Nguyen, Harding, Luong & Rowley, 2021)	Tapley et al. 2021
28	*B. gaolanensis* Song, Wang, Qi, Wang & Wang, 2024	Song et al. 2024
29	*B. gutu* Kuang, Wei & Shi, 2025	Kuang et al. 2025
30	*B. hoanglienensis* (Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018)	Tapley et al. 2018a
31	*B. huangniushiensis* Wang, Wu, Song, Huang, Zhuo & Wang, 2025	Wang et al. 2025b
32	*B. hungtai* Wang, Zeng, Lyu, Xiao & Wang, 2022	Lyu et al. 2023
33	*B. hengshanensis* Qian, Hu, Mo, Gao, Zhang & Yang, 2023	Qian et al. 2023
34	*B. insularis* (Wang, Liu, Lyu, Zeng & Wang, 2017)	Lyu et al. 2023
35	*B. jiangi* (Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020)	Lyu et al. 2023
36	*B. jingdongensis* (Fei & Ye, 1983)	Fei et al. 1983; Lyu et al. 2023
37	*B. jinggangensis* (Wang, 2012)	Lyu et al. 2023
38	*B. jiulianensis* (Wang, Zeng, Lyu & Wang, 2019)	Lyu et al. 2023
39	*B. kuatunensis* (Pope, 1929)	Lyu et al. 2023
40	*B. leishanensis* (Li, Xu, Liu, Jiang, Wei & Wang, 2018)	Lyu et al. 2023
41	*B. lushanensis* Shen, Zhou & Li, 2025	Shen et al. 2025
42	*B. lushuiensis* (Shi, Li, Zhu, Jiang, Jiang & Wang, 2021)	Lyu et al. 2023
43	*B. lichun* Lin, Chen, Li, Peng, Zeng & Wang, 2024	Lin et al. 2024
44	*B. liboensis* (Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017)	Zhang et al. 2017
45	*B. lini* (Wang & Yang, 2014)	Lyu et al. 2023
46	*B. lishuiensis* (Wang, Liu & Jiang, 2017)	Lyu et al. 2023
47	*B. minor* (Stejneger, 1926)	Lyu et al. 2023
48	*B. mirabilis* (Lyu, Wang & Zhao, 2020)	Lyu et al. 2023
49	*B. mufumontana* (Wang, Lyu & Wang, 2019)	Lyu et al. 2023
50	*B. nankunensis* (Wang, Zeng & Wang, 2019)	Lyu et al. 2023
51	*B. nanlingensis* (Lyu, Wang, Liu & Wang, 2019)	Lyu et al. 2023
52	*B. obesa* (Wang, Li & Zhao, 2014)	Lyu et al. 2023
53	*B. ombrophila* (Messenger & Dahn, 2019)	Lyu et al. 2023
54	*B. omeimontis* (Liu, 1950)	Lyu et al. 2023
55	*B. palpebralespinosa* (Bourret, 1937)	Fei et al. 2009; Lyu et al. 2023
56	*B. pepe* (Wang & Zeng, 2024)	Wang et al. 2024
57	*B. puningensis* Wang, Zeng, Lyu, Xiao & Wang, 2022	Lyu et al. 2023
58	*B. qianbeinsis* (Su, Shi, Wu, Li, Yao, Wang & Li, 2020)	Lyu et al. 2023
59	*B. rubrimera* (Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017)	Tapley et al. 2017, 2018b
60	*B. sangzhiensis* (Jiang, Ye & Fei, 2008)	Lyu et al. 2023
61	*B. sanmingensis* (Lyu & Wang, 2021)	Lyu et al. 2023
62	*B. shimentaina* (Lyu, Liu & Wang, 2020)	Lyu et al. 2023
63	*B. shuichengensis* (Tian & Sun, 1995)	Tian and Sun 1995; Tian et al. 2000; Fei and Ye 2016
64	*B. shunhuangensis* (Wang, Deng, Liu, Wu & Liu, 2019)	Lyu et al. 2023

ID	*Boulenophrys* spp.	References
65	*B. spinata* (Liu & Hu, 1973)	Hu et al. 1973; Lyu et al. 2023
66	*B. tongboensis* (Wang & Lyu, 2021)	Lyu et al. 2023
67	*B. tuberogranulata* (Shen, Mo & Li, 2010)	Mo et al. 2010; Fei and Ye 2016; Lyu et al. 2023
68	*B. wugongensis* (Wang, Lyu & Wang, 2019)	Lyu et al. 2023
69	*B. wuliangshanensis* (Ye & Fei, 1995)	Lyu et al. 2023
70	*B. wushanensis* (Ye & Fei, 1995)	Ye and Fei 1995; Fei and Ye 2016; Lyu et al. 2023
71	*B. xiangnanensis* (Lyu, Zeng & Wang, 2020)	Lyu et al. 2023
72	*B. xianjuensis* (Wang, Wu, Peng, Shi, Lu & Wu, 2020)	Lyu et al. 2023
73	*B. xuefengmontis* Lyu & Wang, 2023	Lyu et al. 2023
74	*B. yangmingensis* (Lyu, Zeng & Wang, 2020)	Lyu et al. 2023
75	*B. yaoshanensis* Qi, Mo, Lyu, Wang & Wang, 2021	Lyu et al. 2023
76	*B. yezhongensis* Liu, Feng, Shen, Li, Cheng, Wei, Wang & Su, 2025	Liu et al. 2025
77	*B. yingdeensis* Qi, Lyu, Wang & Wang, 2021	Lyu et al. 2023
78	*B. yunkaiensis* Qi, Wang, Lyu & Wang, 2021	Lyu et al. 2023

verified to be distributed in Ehuangzhang Nature Reserve based on molecular data, which also confirms the expansion of the distribution range of the two species. As the distribution range of the critically endangered *B. acuta* has been exceptionally underestimated, we suggest an update for the extinction risk assessment below.

Additionally, samples taken from five captured adult specimens (GEP a168–170, SYS a009518, and CIB 121751) formed a monophyletic lineage with strong support (BS = 100; BPP = 1.00). This new lineage is a sister taxon to *B. acuta* with strong support (BS = 97; BPP = 1.00) and significant divergence with a mean *p*-distance of 6.5% (Suppl. material 2). Further morphological examination also showed that the corresponding specimens of the new lineage can be distinguished from all recognized congeners. Thus, we describe the corresponding specimens as a new species, which is sympatric with *B. acuta* and *B. yunkaiensis*.

Taxonomic account

Boulenophrys yangchunensis sp. nov.

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Figs 3, 4

English name: Yangchun Horned Toad

Chinese name: yáng chūn jiǎo chán (阳春角蟾)

Type materials. ***Holotype*** • ♂. GEP a170, collected by Jian Wang, Zhao-Chi Zeng, Hong-Hui Chen, Yuan-Hang Li, and Ke Zheng on 11 August 2023 from Guangdong Yangchun Ehuangzhang Provincial Nature Reserve (21.851912°N, 111.421553°E; ca. 1,060 m a.s.l.) in Yangchun City, Guangdong Province, China.

Paratypes. •4 ♂: GEP a168–169, SYS a009518 (field number: GEP a172), and CIB 121751 (field number: GEP a171), same collection data as holotype.

Etymology. The specific epithet *yangchunensis* is in reference to the type locality, Yangchun City.

Diagnosis. (1) small size (SVL 27.6–28.7 mm in five adult males); (2) snout sharpened in dorsal view, canthus rostralis well developed, tongue not notched distally; (3) tympanum distinct; (4) vomerine ridges and vomerine teeth absent; (5) dorsal skin rough and highly granular, discontinuous X-shaped ridge on center of dorsum, discontinuous dorsolateral ridges present, sparse large tubercles on flanks, dorsal limbs with discontinuous transverse ridges and tubercles; (6) outer margin of upper eyelid with a small horn-like prominent tubercle, supratympanic fold distinct and narrow, curving posteroventrally to above arm; (7) two metacarpal tubercles distinct, inner one observably enlarged, relative finger lengths I < II < IV < III, distinct subarticular tubercle at base of each finger; (8) heels just meeting when hindlimbs folded, tibio-tarsal articulation reaching middle to anterior corner of eye; (9) toes without webbing and lateral fringes, inner metatarsal tubercle long ovoid, outer one absent, relative toe length I < II < V < III < IV; (10) dorsal surface yellowish-brown to dark brown, with indistinct irregular dark-brown patches and small orange dots; dark-brown triangular marking between eyes; dorsal limbs and digits light brown with dark-brown transverse bands; and (11) a single nuptial pad bearing dense nuptial spines on dorsal bases of fingers I and II in breeding adult males, subgular vocal sac present in males.

Comparisons. *Boulenophrys yangchunensis* sp. nov. is phylogenetically most closely related to *B. acuta* and *B. gaolanensis* (Fig. 2). However, the new species distinctively differs from *B. acuta* by its heels meeting when hindlimbs are folded (vs. heels not meeting), absence of vomerine ridge (vs. presence of weak vomerine ridge), absence of webbing and lateral fringes on toes (vs. presence of rudimentary webbing and narrow lateral fringes on toes), presence of nuptial pads bearing dense nuptial spines on dorsal bases of fingers I and II in breeding adult males (vs. presence of weak nuptial pads not bearing nuptial spines on dorsal bases of fingers I and II in breeding adult males). The new species further differs from *B. gaolanensis* by its heels meeting when hindlimbs folded (vs. heels not meeting), sharpened snout in dorsal view (vs. rounded snout in dorsal view), presence of nuptial pad bearing dense nuptial spines on dorsal bases of fingers I and II in breeding adult males (vs. nuptial pad and nuptial spines invisible).

Boulenophrys yangchunensis sp. nov. can be easily distinguished from *B. brachykolos*, *B. daoji*, *B. daweimontis*, *B. dongguanensis*, *B. fengshunensis*, *B. frigida*, *B. hungtai*, *B. hengshanensis*, *B. kuatunensis*, *B. lichun*, *B. lushanensis*, *B. nankunensis*, *B. puningensis*, *B. pepe*,

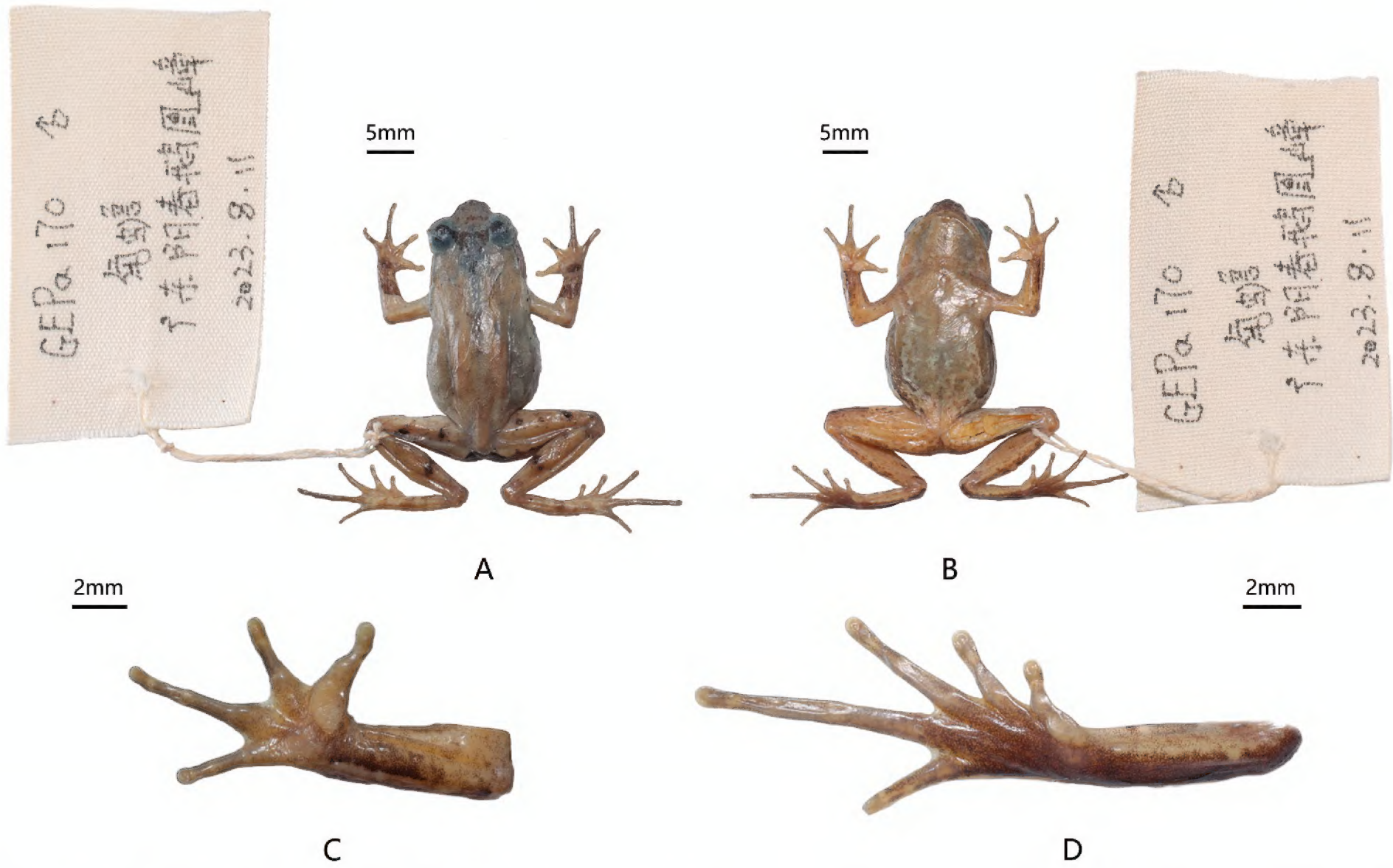

Figure 3. The holotype (GEP a170) of *Boulenophrys yangchunensis* sp. nov. in preservative: dorsal view (**A**); ventral view (**B**); ventral view of hand (**C**); ventral view of foot (**D**). Photos by Jian Wang.

B. insularis, *B. obesa*, *B. ombrophila*, and *B. wugongensis*, by its heels meeting when hindlimbs folded (vs. heels not meeting); and from *B. anlongensis*, *B. baishanzuensis*, *B. binlingensis*, *B. caudoprocta*, *B. cheni*, *B. changyangensis*, *B. congjiangensis*, *B. changyangensis*, *B. chishuiensis*, *B. daxuemontis*, *B. dalaolingensis*, *B. dupanglingensis*, *B. fanjingmontis*, *B. jiangi*, *B. jiulianensis*, *B. jingdongensis*, *B. jinggangensis*, *B. leishanensis*, *B. lushuiensis*, *B. liboensis*, *B. lini*, *B. lishuiensis*, *B. mirabilis*, *B. mufumontana*, *B. nanlingensis*, *B. omeimontis*, *B. palpebralespinosa*, *B. qianbeiensis*, *B. sangzhiensis*, *B. shunhuangensis*, *B. sanmingensis*, *B. shimentaina*, *B. spinata*, *B. tongboensis*, *B. tuberogranulata*, *B. xianjuensis*, *B. yangmingensis*, *B. yezhongensis*, *B. yingdeensis*, *B. yunkaiensis*, and *B. wuliangshanensis* (vs. heels overlapping).

Boulenophrys yangchunensis sp. nov. further differs from *B. angka*, *B. anlongensis*, *B. baishanzuensis*, *B. binlingensis*, *B. elongata*, *B. fansipanensis*, *B. hoanglienensis*, *B. minor*, *B. rubrimera*, *B. shuichengensis*, *B. xiangnanensis*, *B. yaoshanensis*, and *B. yezhongensis* by its absence of vomerine ridge (vs. presence of vomerine ridge); from *B. daiyunensis*, *B. elongata*, *B. fansipanensis*, *B. hoanglienensis*, and *B. rubrimera* by its absence of vomerine teeth (vs. presence of vomerine teeth); and from *B. baolongensis* and *B. boettgeri* by its tongue not notched distally (vs. tongue notched). The new species differs from *B. wushanensis* and *B. gutu* by its absence of lateral fringes on toes (vs. presence of lateral fringes on toes); from *B. yaoshanensis* and *B. caobangensis* by its absence of webbing on toes (vs. presence of webbing on toes); from *B. xuefengmontis* by its tibiotarsal articulation reaching region between middle of eye to anterior corner of eye when legs are stretched forward (vs. between posterior margin of tympanum and posterior corner of eye), and the absence of granules on ventral skin (vs. presence of several granules on chest); from *B. huangniushiensis* by its presence of nuptial pads bearing nuptial spines in males (vs. absence of nuptial pads or nuptial spines).

Description of holotype. Adult male. Body size small, SVL 27.9 mm. Head length almost equal to head width, HDW/HDL 1.00; snout sharpened in dorsal view, projecting, sloping backward to mouth in profile, protruding well beyond margin of lower jaw; top of head flat; eyes moderate in size, ED 0.40 of HDL, pupil vertical, near diamond-shaped; nostril obliquely ovoid; canthus rostralis well developed; loreal region slightly oblique; internasal distance slightly larger than interorbital distance; tympanic region oblique, tympanum distinct and visible in dorsal view; tympanum moderate in size, margin clear, upper margin in contact with supratympanic fold, lower margin in contact with upper lip, TD/ED 0.50; large ovoid choanae at base of maxilla; vomerine ridge and vomerine teeth absent, maxillary teeth present; margin of tongue rounded, not notched distally; presence of single subgular vocal sac.

Forearm length 0.20 of SVL, hand 0.30 of SVL; webbing absent between fingers, lateral fringes absent, relative finger length I < II < IV < III; tips of fingers slightly dilated, round; subarticular tubercles on base of fingers present, distinct; inner metacarpal tubercle

Figure 4. Life aspect of *Boulenophrys yangchunensis* sp. nov.: holotype GEP a170 (**A, B**); paratype GEP a168 (**C**); paratype GEP a172 (**D**). Photos by Jian Wang.

observably enlarged, outer one slightly smaller; single nuptial pad bearing dense nuptial spines present on dorsal surface of first and second fingers, respectively. Hindlimbs moderate in length, tibio-tarsal articulation reaching forward to anterior corner of eye when hindlimb stretched along body; heels just meeting when flexed hindlimbs held at right angles to body axis; crus length 0.50 of SVL and foot length 0.70 of SVL; relative toe length I < II < V < III < IV; tips of toes round and slightly dilated; toes without lateral fringes and webbing; subarticular tubercles on base of toes present and distinct; inner metatarsal tubercle long ovoid and lacking outer metatarsal tubercle.

Dorsal skin rough and highly granular; dense large tubercles on flanks; single horn-like prominent tubercle on edge of upper eyelid; obvious supratympanic fold curving posteroventrally from posterior corner of eye to level above insertion of arm; discontinuous X-shaped ridge on center of dorsum, discontinuous dorsolateral ridges present; dorsal limbs with discontinuous transverse ridges and tubercles; ventral surface smooth; small and distinct pectoral gland closer to axilla; single femoral gland positioned on posterior surface of thigh at midpoint between knee and cloaca.

Coloration of holotype. In life, dorsal surface of body yellowish-brown with indistinct irregular dark-brown patches and small orange dots, dark-brown triangular marking between eyes. A vertical dark-brown band present below eye. Tympanum light brown. Dorsal surface of limbs with dark-brown transverse bands. Tubercles on edge of upper eyelids orange. Supratympanic fold grey white. Iris coppery brown.

In preservative, dorsal surface of body greyish-brown, with markings and patches more distinct. Surface of chest, throat and limbs are yellowish brown, with distinct dark-brown markings and greyish-white patches. Pectoral glands and femoral glands greyish-white.

Variation. Morphometric variations are listed in Table 2. Most of the paratypes are similar to the holotype in morphology and color pattern, except for the following: tibio-tarsal articulation reaching forward to middle of eye when hindlimb stretched along body in the paratype GEP a171; head width larger than head length in the paratype SYS a009518; dorsal coloration yellowish-brown, greyish-brown to dark brown (Fig. 4).

Table 2. Measurements (in mm) of voucher specimens of *Boulenophrys yangchunensis* sp. nov., * holotype.

Voucher	GEP a168	GEP a169	GEP a170*	CIB 121751	SYS a009518
Sex	♂	♂	♂	♂	♂
SVL	28.6	27.6	27.9	28.6	28.7
HDL	10.4	10.1	11.1	10.6	10.4
HDW	10.3	9.7	11.0	10.4	10.6
ED	3.7	3.6	3.8	4.0	3.7
TD	3.5	3.1	3.7	3.5	3.6
TED	3.1	3.0	3.2	3.4	3.3
SNT	3.8	3.7	4.1	3.6	3.5
IND	2.1	2.0	2.0	2.2	2.0
IOD	1.2	1.3	1.1	1.2	1.2
HND	7.8	7.8	7.9	8.1	7.6
RAD	6.0	6.1	6.5	5.9	5.9
FTL	19.9	20.1	20.7	19.7	19.7
TIB	14.0	13.8	14.5	13.3	14.1

Distribution and natural history. Currently, *Boulenophrys yangchunensis* sp. nov. is only known from the type locality, Guangdong Yangchun Ehuangzhang Provincial Nature Reserve, western Guangdong, China. It inhabits flowing montane streams and the nearby forest floor and leaf litter. The habitat is surrounded by evergreen broad-leaved forest mixed with bamboo groves at elevations between 900 and 1,100 m (Fig. 5). Advertisement calls of males were heard from February to May. Males were found calling while perching on leaves that are 10–30 cm above the ground.

Conservation status of relevant *Boulenophrys* species in Guangdong

Recommendation for extinction risk assessment for *Boulenophrys acuta*

Distribution: Heishiding Nature Reserve, Fengkai County, Zhaoqing, Guangdong Province, China (Li et al. 2014; Lyu et al. 2023); Mt. Yunkai, Xinyi City, Guangdong Province, China (Song et al. 2024); Guangdong Yangchun Ehuangzhang Provincial Nature Reserve, Yangchun City, Guangdong Province, China (this study).

Extinction risk assessment: based on the analysis on GeoCAT, we determined an AOO of 3,170 km^2 and an AOO of 12 km^2. As the species is found at three independent locations (to assess against criteria B2a), and there is a continued observed decline in the (iii) quality of the habitat, we recommend the species be listed as Endangered following the criteria B1ab(iii) and B2ab(iii). If relying on the criteria D (very small population and/or restricted distribution) for the recommendation of assessment, the species would be listed as Vulnerable D2 because the AOO is smaller than 20 km² and the species occurs at fewer than five locations. This recommendation for assessment is a non-genuine downlisting (ASG

Figure 5. Microhabitat of *Boulenophrys yangchunensis* sp. nov. in Guangdong Yangchun Ehuangzhang Provincial Nature Reserve, Yangchun City, Guangdong Province, China. Photos by Bin-Bin Zhan.

2021), from Critically Endangered (CR) to Endangered (EN), because of the discovery of two new populations.

Recommendation for extinction risk assessment for *Boulenophrys gaolanensis*

Distribution: Gaolan Island, Jinwan District, Zhuhai City, Guangdong Province, China (Song et al. 2024; Jian Wang, pers. comm.).

Extinction risk assessment: based on the analysis on GeoCAT, we determined an AOO of 0 km^2 and an AOO of 8 km^2. As the species is found at two independent locations (to assess against criteria B2a), and there is a continued observed decline in the (iii) quality of the habitat, we recommend the species be listed as Critically Endangered following the criteria B1ab(iii) and B2ab(iii). If relying on the criteria D (very small population and/or restricted distribution) for the recommendation of assessment, the species would be listed as Vulnerable D2 because the AOO is smaller than 20 km^2 and the species occurs at fewer than five locations. This recommendation for assessment as Critically Endangered (CR) has the potential to be updated through a non-genuine downlisting, as there are still a large number of potential distribution areas in the adjacent coastal mountainous regions.

Recommendation for extinction risk assessment for *Boulenophrys yunkaiensis*

Distribution: Mt. Yunkai, Xinyi City, Guangdong Province, China (Qi et al. 2021); Guangdong Yangchun Ehuangzhang Provincial Nature Reserve, Yangchun City, Guangdong Province, China (this study).

Extinction risk assessment: based on the analysis on GeoCAT, we determined an AOO of 29.8 km^2 and an AOO of 8 km^2. As the species is found at two independent locations (to assess against criteria B2a), and there is a continued observed decline in the (iii) quality of the habitat, we recommend the species be listed as Critically Endangered following the criteria B2ab(iii). If relying on the criteria D (very small population and/or restricted distribution) for the recommendation of assessment, the species would be listed as Vulnerable D2 because the AOO is smaller than 20 km^2 and the species occurs at fewer than five locations. This recommendation for assessment as Critically Endangered (CR) has the potential to be updated through a non-genuine downlisting, as the species is so far known to occur on two disconnected mountain ranges adjacent to likely suitable habitat.

Recommendation for extinction risk assessment for *Boulenophrys yangchunensis* sp. nov.

Distribution: Guangdong Yangchun Ehuangzhang Provincial Nature Reserve, Yangchun City, Guangdong Province, China (this study).

Extinction risk assessment: based on the analysis on GeoCAT, we determined an AOO of 0 km^2 and an AOO of 4 km^2. As the species is found at a single location (to assess against criteria B2a), and there is a continued observed decline in the (iii) quality of the habitat, we recommend the species be listed as Critically Endangered following the criteria B1ab(iii) and B2ab(iii). If relying on the criteria D (very small population and restricted distribution) for the recommendation of assessment, the species would be listed as Vulnerable D2 because the AOO is smaller than 20 km^2 and the species occurs at fewer than five locations. This recommendation for assessment as Critically Endangered (CR) has the potential to be updated through a non-genuine downlisting if the species was present in the continuous habitat likely to be suitable to support a larger population. However, the continuous and likely suitable area is less than 400 km^2, placing the species in the Endangered (EN) category under the current threat conditions even if it was present throughout the whole area, which surveys have already demonstrated not to be accurate.

Discussion

The genus *Boulenophrys* is predominantly distributed in China with notable diversity (75 of 79 species, approximately 95%; Frost 2025; this study). However, accurate conservation assessments for this genus are hindered by morphological conservatism, sympatric distribution patterns, and inadequate follow-up field surveys. Convergent traits, or intraspecific variation, can obscure true species boundaries as they result in misidentifications (Lyu et al. 2023; Lin et al. 2024; Song et al. 2024).

The morphological crypticity in *Boulenophrys* species highlights the limitations of traditional taxonomy and the significance of applying technologies such as molecular identification (Liu et al. 2018). Multiple *Boulenophrys* species often coexist in the same habitats (Lyu et al. 2023). This sympatry further complicates species identification and ecological niche differentiation, potentially leading to the underestimation of extinction risks for rare or microendemic taxa. Furthermore, the lack of follow-up surveys and adequate species identification significantly undermines the accuracy of distribution data and the resulting threat assessments. Specifically, these shortcomings result in delayed recognition of threats, delayed acknowledgements of population declines, absence of a baseline to calculate range contraction, miscalculation of distribution range, oversight of emerging threats, inaccurate species delimitation, and inefficient conservation prioritization (Lin et al. 2024; Zeng et al. 2024).

These challenges mentioned above collectively cause delays in the recognition of threatened species and the underestimation of extinction risks. The conservation of *Boulenophrys* species demands urgent action to address taxonomic uncertainties and survey gaps in taxonomy, distribution, threat assessment, and habitat degradation (e.g., Borzée 2023). Thus, we suggest the following management suggestions for conservation: (1) strengthen molecular systematics (integrating DNA barcoding and phylogenetic analyses to resolve cryptic species complexes and species identifications) and rely on whole genome sequencing when possible; (2) prioritize targeted field surveys (conducting intensive surveys in less-visited areas and using standardized protocols—e.g., environmental DNA sam-

pling and acoustic monitoring—to detect rare or elusive species); (3) expand the protected area networks (establishing new reserves and buffer zones in regions with high *Boulenophrys* diversity and ensuring connectivity between habitats to mitigate fragmentation effects) through the use of all types of protected areas, including microreserves as they are a good fit for amphibians (Volenec and Dobson 2020); (4) promote long-term monitoring (implementing DNA barcoding databases and qualitative PCR on eDNA to detect drops in population size); and (5) address habitat threats (mitigating habitat loss by enforcing stricter land-use regulations and restoring habitat in critical areas).

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Supplementary material 1

Collection localities, voucher data, and GenBank accession numbers for all samples used in this study

Authors: Yi-Yang Zhao, Shi-Shi Lin, Yuan-Hang Li, Zhi-Tong Lyu, Ke Zheng, Zhao-Chi Zeng, Amaël Borzée, Jian Wang

Data type: xlsx

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Supplementary material 2

Genetic distances between members of *Boulenophrys* (uncorrected *p*-distance) in 16S rRNA sequences

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Data type: xls

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